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**The failure of anxiolytic therapies in early clinical trials:
what needs to be done?**

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Abstract

Introduction: Anxiety spectrum disorders (ASDs) are highly prevalent psychiatric illnesses that affect millions of people worldwide. Strongly associated with stress, common ASDs include generalized anxiety disorder, panic, social anxiety, phobias and drug-abuse related anxiety. In addition to ASDs, several other prevalent psychiatric illnesses represent trauma/stressor-related disorders (TSRDs), such as post-traumatic stress disorder (PTSD) and acute stress disorder.

Areas covered: Anxiolytic drugs, commonly prescribed to treat ASDs and TSRDs, represent a highly heterogeneous group, modulating multiple neurotransmitters and physiological mechanisms. However, overt individual differences in efficacy and the potential for serious side effects (including addiction and drug interaction) indicate a need for further drug development. Yet, over the last 50 years, there has been relatively little progress in the development of novel anxiolytic medications, especially when promising candidate drugs often fail in early clinical trials.

Expert opinion: Here we present recommendations of the Task Force on anxiolytic drugs of the International Stress and Behavior Society (ISBS) on how to improve anxiolytic drug discovery. These recommendations cover a wide spectrum of aspects, ranging from methodological improvements (automation, biomarker validation, dose/test/sex/age/strain selection) to conceptual insights ('clinization', targeting disorder spectra and neurodevelopmental trajectories) and innovation (focusing on novel pathways and imaging ASD pathogenesis).

Keywords: anxiolytic drug, model organism, experimental model, preclinical study, clinical trial

Article Highlights:

- Anxiety spectrum disorders (ASDs) affect millions of people worldwide
- Many candidate drugs for treating ASDs fail in preclinical or early clinical trials
- Here, we propose several methodological recommendations to improve ASD drug discovery
- Conceptual recommendations include focus on disorder spectra and developmental trajectories
- Focus on novel biomarkers and pathways is critical for enhancing anxiolytic drug development

1. Introduction

Anxiety spectrum disorders (ASDs) are highly prevalent psychiatric conditions that affect millions of patients worldwide¹⁻³. In addition to generalized anxiety disorder (GAD), other common ASDs include panic disorder, social anxiety, agoraphobia, specific phobias and drug-related anxiety⁴ (Fig. 1). The trauma- and stressor-related disorders (TSRDs), such as post-traumatic stress (PTSD) and acute stress disorders⁴, also represent serious debilitating neuropsychiatric illnesses, which (like ASDs) are pathogenically triggered by stress (Fig. 1).

Anti-anxiety (anxiolytic) drugs, commonly prescribed to treat ASDs and TSRDs (Table 1), are currently one of the most prescribed classes of psychotropic medication^{5,6}. While benzodiazepines and selective serotonin reuptake inhibitors (SSRIs) remain common ‘traditional’ anxiolytic drugs (Fig. 1), several other drug classes are currently used to treat ASD, collectively representing a heterogeneous group that targets a wide spectrum of CNS pathways (Table 1). The availability of novel potent anxiolytics is critical for combating the growing societal and mental health impacts of ASDs⁷. For example, novel efficacious anxiolytics are particularly needed for treatment-resistant ASD patients, and those with complex comorbid disorders (e.g., substance addiction, traumatic brain injury, developmental disorders and dementia), all of which factor into individual variability in the effectiveness of anxiolytic compounds. With the advent of inexpensive whole-genome arrays (with the remarkable potential for patient-tailored therapies), the need for rapid testing of novel compounds is even more apparent. For instance, subtypes of anxiety and mood disorders, and even specific symptoms, such as anhedonia, respond differentially to anxiolytic compounds even as they may be described in a patient under the umbrella term ASD. Thus, the discovery of novel anti-anxiety agents becomes urgently needed to ensure efficient (and, eventually, disorder-specific) treatments of ASDs⁸.

Anxiolytic drug development, like with other classes of drugs, follows the general research ‘pipeline’, as summarized in Fig. 2. Before an anxiolytic drug can go to clinical trials, there are

multiple currently available *in-vitro* and *in-vivo* animal (pre-clinical) models, which are well established and successfully applied to CNS drug discovery⁹. Comprehensively evaluated in recent literature^{10, 11}, their strengths and limitations will not be discussed here. However, as the drugs move from pre-clinical to clinical trials, one of the main recognized challenges is a frequent failure of anxiolytic drugs in early clinical trials^{11, 12}. In response to this growing biomedical problem, the International Stress and Behavior Society (ISBS) has established the Strategic Task Force (chaired by Dr. Allan V. Kalueff) on anxiolytic drugs, comprised of recognized international experts in the field. The present paper outlines the ISBS Task Force recommendations, aiming to improve the success of anxiolytic drugs in early clinical trials.

2. Recommendations of the ISBS Strategic Task Force on anxiolytic drugs

Given multiple conceptual and methodological limitations in modeling complex human brain disorders in animals^{9, 13, 14}, we recognize that targeting the emotional aspects of ASDs is particularly difficult, and that many subjective emotional ASD-related states may not exist in animals. Likewise, humans possess unique socio-cultural features which cannot be modeled in animal preclinical models, but yet are important modulatory factors in the etiopathology and efficacious treatment of ASDs. Thus, the experimental manipulations widely used in animal models, ranging from behavioral to genetic and pharmacological challenges, may be unable to fully recapitulate similar factors in humans⁹. However, despite these limitations, animal models continue to be indispensable for studying neurobiology of ASD and anxiolytic drug discovery^{11, 15-21}. Several strategies summarized here will address the problem of enhancing anxiolytic drug discovery and improving the success of their transition from preclinical to early clinical trials.

2.1. Methodological recommendations

- **Focus on multiple anxiety-related endophenotypes**

Modeling complex human brain disorders, such as ASDs or TSRDs, is commonly performed by deconstructing them into ‘smaller’ units, termed behavioral and physiological endophenotypes (see ^{22, 23}

for details). Endophenotypes are usually clustered within various distinct ‘bigger’ behavioral domains, such as affective, cognitive, social, motor or reward sensitivity^{22, 23}. Because most currently available anxiety behavioral tests are traditionally targeting exploratory domain^{9, 14, 24}, current preclinical studies of ASDs appear to be generally biased towards this particular endophenotypic domain. Here, we emphasize the importance of in-depth focusing on other ASD-related endophenotypes (and their sensitivity to anxiolytic drugs) as well. For example, the social interaction test may inform us on the preclinical model’s ability to detect agents to treat social anxiety, whereas drugs improving sensorimotor integration (e.g., assessed in the Suok test¹⁴), may be relevant to detecting agents to treat agoraphobia.

- **Consider sex and age as an important variable in preclinical and clinical trials**

Although a major improvement has been achieved towards balancing the sample proportions of men and women in human clinical trials, basic biomedical research is still lagging behind, especially as ‘over-represented’ male animals and cells may obscure key sex differences that may inform clinical studies²⁵. With various ASDs and TRSDs showing clear sex differences in both the symptoms and epidemiology, the inclusion of both sexes in preclinical studies of anxiety may contribute to improving anxiolytic drug discovery. As these candidate agents move toward the early clinical trials, we consider sex as a biological variable able to affect the reproducibility, validity and generalizability of research findings. Likewise, the majority of preclinical in-vivo studies are routinely performed in adult animals. However, according to the US Census Bureau report (<http://www.census.gov/cgi-bin/broker>), approximately 25% of the 2014 World global population are children <14 years old, and nearly 25% are older adults > 50 years old. Therefore, with adults representing only ~50% of the global population, further inclusion of younger and older animal groups is recommended in ASD and anxiolytic drug research, in order to adequately reflect the current status of human population.

- **Use both inbred and outbred strains for ASD research**

The discussion of what animal strains to use is an important aspect for improving translational neurobehavioral ASD research and anxiolytic drug development²⁶⁻²⁸. With the growing availability of mouse, rat or zebrafish strains for neuroscience research, the vastly predominant practice is to use selected inbred strains for drug screening assays, thereby ensuring a better genetic controllability of the research design. Here we note, however, that the global human population is highly heterogeneous genetically, representing an important ‘populational’ factor for clinical trials. Therefore, a more balanced, complementary use of both inbred and outbred animal strains in CNS research and drug discovery may lead to more validity metrics of treatment, side-effects, and contraindications. Termed ‘population validity’ in a recent literature²⁹, the focus of this strategy is on the ‘demographic’ aspects of drug action, in addition to examining its overall effect size and direction. In other words, a weaker drug that affects a larger spectrum of an animal sample similarly, may be preferred as a candidate for future clinical trials, versus more robust and potent agents that are more subject-selective.

- **Consider the role of test batteries**

One of the key problems in neurobehavioral research, which becomes particularly relevant to modeling ASDs, is the standardization of behavioral tests and procedures³⁰⁻³². For example, while it is important for consistency and cross-laboratory comparability of data, novel drugs may have unique profiles of action, which may require more specific models and screens¹¹. Furthermore, given the well-known impact of test batteries on animal ASD-related behavior^{26, 27, 33}, preclinical studies of novel ASD drugs may benefit from more active inclusion of modifications of experimental procedures and test batteries, to dissect this aspect in decoding drug-evoked profiles³⁴.

- **Consider a wider (clinically relevant) spectrum of drug regimens**

In line with the previous recommendation, we also note that clinical practice typically employs a wide spectrum of drug regimens and examines multiple therapeutic outcomes, depending on time of treatment initiation, treatment duration, frequency, and treatment assessment time (Table 3). Accordingly, similar approaches can be applied to drug screening during the preclinical phase using

animal models. Thus, while the majority of ASD studies are traditionally limited to acute or chronic drug treatment protocols, a standardized preclinical test battery can include a wider spectrum of regimens, similar to that presented in Table 2. The increased amount of labor (as compared to merely using acute and chronic drug treatments) can be offset by improving high-throughput of such testing by automating: 1) drug/stimuli delivery, 2) behavior analyses (e.g., using video-tracking, behavior-recognition and/or 24-h monitoring) and 3) animal handling and other experiment-related procedures.

- **Optimize genetic models**

Genetically modified (e.g., selectively bred, mutant or transgenic) models are widely used in anxiety research²⁶⁻²⁸. For example, there are multiple inbred, mutant or transgenic mouse strains with anxiety-like and anxiolytic-like phenotypes currently listed in the Mouse Phenome Database (MPD)³⁵ and Mouse Genome Informatics (MGI) databases. However, it is becoming increasingly recognized that allelic and expression variation contribute to variance in the resultant CNS phenotype. Therefore, better genetic animal models of human disorders are needed in order to address this complexity, rather than drawing conclusions from traditional knockout studies (which currently remain the ‘mainstream’ pharmacogenetic approach in anxiolytic drug discovery). The latter includes a wide spectrum of genetic manipulations, such as transgenic models, gene silencing with morpholinos, as well as the use of ‘humanized’ animals (with human genes being inserted into their genomes). We recommend including a wide spectrum of such novel emergent genetic models in anxiolytic drug discovery, to complement traditional ‘knockout’ models, currently still dominating pharmacogenetics.

- **Combine behavioral data with physiological biomarkers**

In addition to comprehensive behavioral analyses, the importance of developing novel biomarkers of brain disorders (including depression, ASDs and other affective disorders) has recently been recognized in the literature^{36, 37}. A range of biological markers can be used to evaluate stress/ASD-related states in preclinical models, including corticosteroids³⁸⁻⁴⁰, neuropeptide Y (NPY), proopiomelanocortin (POMC) and adrenocorticotrophic hormone (ACTH)⁴¹. Thus, although biological

markers are not yet approved as part of the diagnostic criteria for ASDs, they may help predict the potential disease trajectory, and support decisions for specific early therapeutic and prophylactic measures³⁷. Other physiological biomarkers relevant to ASD include electroencephalographic (EEG) responses, REM sleep waves, and sensorymotor integration. Overall, the need to validate and discover new predictive markers of ASDs becomes important for developing more valid novel preclinical models for anxiolytic drug discovery.

- **Combine behavioral data with immunological phenotypes**

Various cytokines, such as interleukins (IL) IL-1, IL-6, and tumor necrosis factor TNF- α ^{7, 42-49}, have been suggested as mediators of stress and ASD in a wide range of species, from fish to humans⁵⁰. Both clinical and experimental studies have demonstrated that inflammation in the peripheral immune system and/or in the brain can induce anxiety-like behavior and neuroendocrine changes in patients or animal models⁵⁰. The mechanisms by which inflammation induces anxiety include stimulating the hypothalamic-pituitary-adrenal axis to release corticotrophin-releasing hormone (CRH, which triggers glucocorticoid secretion) and enhancing the activity of the indoleamine 2,3-dioxygenase (IDO) enzyme, which is the first rate-limiting enzyme of the tryptophan degradation pathway. The increased tryptophan degradation can induce serotonin depletion and anxious mood. On the other hand, the downstream metabolites from this pathway, such as 3-hydroxykynurenine, quinolinic acid and kynurenic acid, are neuroactive metabolites which can modulate several neurotransmissions, such as glutamatergic, GABA-ergic, dopaminergic and noradrenergic neurotransmissions. Furthermore, by activating microglia and oxidative stress, such processes may damage neurons and cause dysfunction of neurotransmitter system. Recently, anti-inflammatory drugs or omega-3 fatty acids, such as COX2 inhibitors and eicosapentaenoic acid, have been shown to exert anxiolytic effects in animal model of depression or IL-1 β -induced neuroinflammation model, acting via inhibiting inflammation and glucocorticoids⁵¹⁻⁵⁴. Therefore, not only neuroimmune biomarkers can be used to improve the validity of ASD models, but anti-inflammatory drugs can also be a group of candidates for the development of

new anxiolytic treatments. Here, we call for a wider application of neuroimmunological models in both basic ASD research and anxiolytic drug discovery.

- **Clarify and rethink inclusion/exclusion criteria**

In general, inclusion and exclusion criteria represent recognized standards necessary to determine whether a subject can or cannot be allowed to participate in a clinical trial. Universally used in clinical studies, these criteria are critical for ensuring generalizability and reliability of trial data⁵⁵. Clinical studies are also carefully controlled for various subject-related factors, such as sex, race, age and/or social group (e.g., NIH guidelines on targeted/planned enrollment, http://grants.nih.gov/grants/funding/women_min/guidelines_amended_10_2001.htm). In contrast, as already mentioned, animal ASD research is typically conducted in homogenous (often same-sex/age inbred) cohorts, and its exclusion/inclusion criteria not only differ fundamentally from clinical criteria, but also vary considerably, with almost no accepted or rationalized standards existing in the literature. One can argue that ‘data is data’, strongly advocating for a total inclusion in data analyses of all animal subjects used. Others may utilize ‘down-to-the-earth’ approach, routinely applying a wide range of exclusion criteria, ranging from trivial (e.g., missed injection, data input error, animal dropped from the apparatus during testing) to rather exotic (e.g., a female rat found in a male cage, a former employee destroyed the data; NB: references not provided here on purpose). Removal of outliers is also often performed, based on phenotypes demonstrated (e.g., removing overly active or inactive animals), but without consideration of potential stratification in population’s responses to anxiolytic agents. Although our panel does not provide here a set of guidelines for using exclusion/inclusion criteria in basic ASD/anxiolytic research, we welcome further critical thinking and multidisciplinary discussion of this problem. Importantly, exclusion and inclusion criteria in animal preclinical trials must be explicitly stated and justified in the reported studies, to ensure consistency and reliability in data interpretation and analyses. Finally, in order to improve the anxiolytic drug pipeline, it may be useful to apply

conceptually similar exclusion/inclusion criteria across pre- and clinical studies, to ensure that data can be more reliably compared and evaluated.

2.2. Conceptual recommendations

- **Focus on negative effects of putative anxiolytic compounds: ‘clinization’ of preclinical trials**

Understanding the reasons why early clinical trials often fail is critical to developing new anxiolytic drugs. Fundamentally, there are key differences between the goals of clinical and preclinical trials. For example, while the preclinical drug discovery phase is driven by innovation and focus on mechanistic insights, clinical trials mainly aim to evaluate and minimize unwanted side effects, some of which may or may not be detected during the animal screening (Fig. 2). Thus, the drugs that appear to be promising in preclinical trials (i.e., because of their strong ‘desired’ effects) may later be rejected in clinical trials due to the magnitude of unwanted side-effects. To address this problem conceptually, we suggest a ‘clinization’ strategy, in which it would be valuable to include ‘clinical-like’ features and focus on standardized assessment of typical clinical side-effects, in addition to searching for compounds with the most robust anxiolytic profile). For instance, benzodiazepines are potent anxiolytics in animals and humans, but their unwanted side effects include sedation, cognitive deficits and addiction. Thus, an increased separation of anxiolytic profile from sedative, hypnotic, addictive or cognitive effects may be introduced as a standard requirement (rather than an undesired outcome) for evaluating potential anxiolytics during the preclinical phase. Likewise, SSRIs are globally used to treat ASD, but may trigger serotonin toxicity (serotonin syndrome), if overdosed or combined with other psychotropic drugs^{56, 57}. From this point of view, rather than relying mainly on the strength of desired anxiolytic effects, the decision to select a lead compound for future early clinical trials may be based on carefully balancing its ‘pros’ (anxiolytic potential) with ‘cons’, assessed in a battery of specialized ‘side effect’-oriented screens (Fig. 2). In other words, a less potent but safer drug may be

preferred for moving to early clinical trials (over a more potent but more hazardous agent, identified during the preclinical trials).

- **Target the disorder spectra**

Mounting evidence indicates that brain disorders often have a spectrum nature, which is reflected in their overlapping genetic determinants, molecular pathways and symptoms⁵⁸⁻⁶⁰. We have argued previously that targeting the spectrum nature of CNS disorders in animal models is an important strategy to complement the traditional, often mono-symptom models^{61, 62}. This notion becomes particularly critical for modeling ASDs, often comprised of multiple symptoms (e.g., social impairment and anxiety in social anxiety, anxiety and motorisensory deficits in agoraphobia). Accounting for the spectrum nature of stress-related CNS disorders is also important because of heavily overlapping comorbidities in many psychiatric disorders⁶³⁻⁶⁵. One logical approach is to implement domain-oriented strategies and integrate multiple domains of these disorders as a system, focusing on modeling such overlaps in animals^{25, 26}. To the best of our knowledge, no anxiolytic drugs have been tested to specifically target the pathogenic overlap between different symptoms of ASDs, and this gap remains to be filled as we translate preclinical models to clinical trials. Using this integrative approach is also expected to empower high-throughput animal models and to facilitate discovery of new biomarkers, behavioral phenotypes and drugs⁶¹.

- **Widen the spectrum of model organisms**

Increasing the spectrum of model organisms to mimic anxiety pathogenesis and screen novel anxiolytic drugs is recognized as an important strategy in biological psychiatry⁹, and the ISBS has been a long-term proponent of these views. Surprisingly, many laboratories remain 'loyal' to their favorite model species, making multi/cross-species research a rather rare and risky academic endeavor⁹. While rodents continue to be the most commonly used animal model in the pre-clinical testing phase of novel anxiolytics⁶⁶, such models can often be limited by potentially lower throughput and high cost of husbandry. From this point of view, alternative model organisms, such as zebrafish, become

particularly useful due to their high-throughput vertebrate nature, high genetic or physiological homology to humans and rodents, as well as low cost, which potentially allows for higher-throughput testing^{15, 18, 67}. Like rodents, zebrafish have overt and easily quantifiable behaviors¹⁶ and an endocrine (hypothalamic-pituitary-interrenal, HPI) axis homologous to the human HPA axis⁶⁸. Due to their high throughput and genetic tractability, zebrafish became a promising new model species for studying various brain disorders, including anxiety⁶⁹, and can be used here as an example of how including alternative (preferably vertebrate) model species to complement traditional preclinical drug screening in rodents can optimize and enhance anxiolytic drug discovery. For instance, zebrafish have well-developed GABA-ergic system, and are generally highly sensitive to benzodiazepines (own unpublished studies). Likewise, the relative potency of psychotropic drugs is often similar across species, from fish to rodents to humans⁷⁰, strongly supporting the value of using alternative (and potentially more high-throughput) species for anxiolytic drug development.

- **Include peripheral metrics of anxiolytic efficacy**

One of the problems recognized by the ISBS Strategic Task Force is that anxiolytic drugs are traditionally viewed as psychotropic medications ('brain pills') to specifically target ASDs. The anxioreactivity of anti-anxiety drugs is, of course, an important goal in ASD drug discovery⁷¹. However, while anxiety is a cognitive-affective state, it does not exist independently from the periphery. Indeed, various physiological processes that occur outside CNS play a key role in brain responses to stress. For example, aberrant endocrine or cytokine stress responses or disordered metabolic mechanisms have long been associated with ASDs. Thus, novel drugs that correct dysregulated peripheral hormonal or immune physiological mechanisms must be considered as a potential new strategic direction for anxiolytic drug discovery. A strong argument in support of this approach is the fact that peripheral biomarkers are the most clinically available measures in patients, therefore increasing projectability of our recommendation. Stemming from 'integrative' systems biology-based views of disease pathogenesis, it is logical to expect that developing anxiolytics 'for the

body' may complement the search for novel anxiolytics 'for the mind', or even work synergistically to correct both body and mind in parallel.

- **Focus on neurodevelopmental trajectories of ASD**

In line with the recognized need to target a wider spectrum of subjects' age groups, mounting evidence indicates that brain disorders, such as ASDs, often have neurodevelopmental trajectories, as the disordered mechanisms start much earlier than the disease manifests itself clinically^{72, 73}. For example, the accumulated literature on anxiety-related effects in adults following adolescent or neonatal drug treatments is a popular area of rodent ASD research^{74, 75}. In line with this, *in-vivo* screens in embryonic and larval zebrafish are becoming increasingly popular for studying developmental disorders, as they combine the efficiency of high-throughput methods with the rapid growth of larval zebrafish⁷⁶. In summary, regardless of what the model organism is used to study novel anxiolytic drugs, a stronger focus on neurobiological origins of early brain deficits, as well as studying their delayed effects later in life, is recommended to ensure a high validity and translatability of preclinical anxiolytic screening data. We propose an additional sub-criterion of validity – neurodevelopmental validity – which can be applied to animal model development in the field of ASD research (and beyond), as part of their construct validity. Thus, the animal model will have high neurodevelopmental validity if its developmental trajectories mimic those observed for the disorder clinically. Accordingly, anxiolytic drug screens that show high neurodevelopmental validity will most likely be efficient in detecting clinically relevant pathogenic trajectories in humans. Applying this approach to achieve two goals - 1) enhance the efficacy of anxiolytic drug screening (by correcting the critical 'developmental' trajectories of CNS pathogenesis) and 2) minimize clinically relevant neurodevelopmental side-effects of anxiolytic drugs - is expected to markedly improve the success of anti-ASD drug development.

- **Moving along (and against) the traditional drug development pipeline**

One of the general views on drug development process is comparing it with a pipeline (Fig. 1), in which the discovery goes gradually from preclinical to clinical trials. However, this is not always the

only direction, as, for example, some approved drugs can be ‘repurposed’ and used off-label (Fig. 2). Therefore, it is likely that additional preclinical studies of animal anxiety can be particularly helpful to identify novel anxiolytic compounds among drugs already developed for treating other disorders⁷⁷. Another situation when moving ‘against the pipeline’ from clinical to preclinical studies can be useful is the field of complementary and alternative medicine (CAM, Table 2). For instance, multiple medicinal plants have been used historically to treat various mental conditions, and this rich clinical evidence can now be re-assessed by using animal models to better dissect various molecular mechanisms of such action. Thus, we argue that moving the drug along the drug development pipeline in both directions (preclinical -> clinical; clinical -> preclinical) can be beneficial for innovative anxiolytic drug discovery (Fig. 2).

2.3. Fostering innovation and discovery

- **Focus on novel putative pathways**

Recognizing the spectrum nature of CNS pathogenesis⁵⁸⁻⁶⁰, in our view, it is critical to search for novel putative pathways of anxiolytic drug action, in addition to targeting their traditional, well-established mechanisms (Table 1). For example, a recent ‘interlinking genes’ concept has been suggested⁷⁰ to improve our understanding of ASDs and other brain disorders (also see discussion on complex, spectrum nature of CNS pathogenesis above). This concept postulates that novel, currently unrecognized class of functional gene products and encoded molecular pathways that likely contribute to the pathogenesis of brain disorders, based on shared molecular pathways and interacting mechanisms⁷⁰. While not influencing the phenotypes per se, such novel genes can modulate the overall interrelatedness of several disordered phenotypes⁷⁰. Accordingly, a principally new class of anxiolytic drugs, targeting pathways controlled by these novel proposed genes, may be developed to treat ASDs by disrupting pathways that synchronize and synergize anxiety endophenotypes. Currently not ‘on the radar’ of drug developers (because of the lack of direct effects on anxiety symptoms – the main current focus of interest of preclinical or clinical trials), such novel drugs may become an important new class

of anxiolytic medications (Fig. 2). Targeting the proposed novel pathways and principally new mechanisms, this approach may be applied toward the discovery of new drugs to treat ASD and other related brain disorders⁷⁰. For example, ‘disconnecting’ social behavioral domain from anxiety may be used to treat social anxiety. Likewise, pharmacogenic decoupling of vestibular and emotional deficits may be relevant to treating agoraphobia, whereas separating drug abuse- from anxiety-related mechanisms may lead to effective anti-withdrawal anxiolytic therapy. Finally, in addition to targeting the well-established mechanisms of anxiolytic drug action *per se* (e.g., via its receptors or transporters), it is important to ensure the robust efficacy of the drug. Thus, novel anxiolytic drugs can be developed based on targeting the drug efficacy predictors, in addition to modulating more traditional drug targets, such as various CNS receptors or transporters (Fig. 4).

- **Apply modern imaging technologies to anxiolytic drug discovery**

Multiple behavioral tests are currently available to improve and refine research in the field of ASDs^{14, 18, 78, 79}. Several challenges, however, exist, and include discriminating ASD-like behaviors from depression-like, autism-like, psychotic-like or Parkinson’s-like symptoms. For example, their symptom clusters and even models/tests can appear similar, but could have quite different origins (e.g., consider pollution in the lake: is the problem in the well spring, the riverbank, or the lake itself?). However, discriminating among such types of behavior may become possible by using video-tracking software, latest neuroimaging tools, as well as analyzing physiological biomarkers, and genomic/epigenetic mechanisms. Likewise, optogenetic tools have been particularly used to link neural circuits to various behavioral and social phenotypes relevant to ASD^{80, 81}. In addition, various imaging techniques, such as magnetic resonance imaging and diffusor tension imaging, have also been used to study neural circuitry in humans and animal models of ASD^{82, 83}. Combined with existing behavioral and social paradigms, these important imaging methodologies will further foster ASD research, and their more active use for anxiolytic drug screening (in both animals and humans) must be encouraged.

3. Conclusion

Developing novel anxiolytic drugs is a difficult but important task. One of key challenges in developing a valid animal model for ASDs, recognized by our panel of experts, is differentiating between distinct stress-related (e.g., anxiety, fear, panic and other affective, such as depression-like) states, as well as dissecting them from global changes in animal motor activity. For example, hypolocomotion can often be observed in relation to anxiety and depression⁸⁴, and better diagnostic acuity between various behavioral symptoms is therefore necessary for further improving anxiolytic drug discovery. Evaluating a spectrum of complex behavioral phenotypes in response to novel drugs represents another key, but related, methodological problem. Therefore, novel efficient paradigms developed must be able to discriminate between actual anxiety-like phenotypes and other, nonspecific side-effects (e.g., sedation, cognitive deficits or seizures).

One of the key challenges in the field of biological psychiatry is the amount of clinical and preclinical results, influencing collecting meaningful biological information using this ‘big data’. However, our ability to analyse large amounts of biological data becomes possible due to a rapidly increasing computer processing power and a wider application of powerful biostatistical tools. Furthermore, with the growing utility of bioinformatics⁸⁵ and systems biology⁸⁶ in drug discovery, various phenomics-oriented approaches become critical for linking together brain disease states across various species and domains (often generating new insights by revealing novel patterns not easily seen without the ‘bigger picture’ approaches)^{87,88}. For example, combining data from proteomic and genomic studies over multiple model organisms can help uncover shared pathways and behaviors in psychiatric diseases, such as ASDs. Therefore, we welcome a more active application of *in-silico* data-mining and neuroinformatic approaches^{89,90} for uncovering novel pathways and identifying new anxiolytic drug targets. Importantly, these advanced tools will enable moving beyond overt symptom manifestations and allow for elucidating the underlying mechanisms that trigger the symptoms. Finally, we encourage a cross-disciplinary dialogue and multi-level conceptualization of anxiety disorders, their clinical symptoms, neural circuits, molecular networks and the existing animal models. Several

important, thoughtful papers published recently can be particularly recommended for further discussion on experimental anxiety models, their validity, applications for anxiolytic drug discovery⁹¹⁻⁹⁵ and cross-species phenomics⁹⁶, as well as the role of behavioral and cognitive factors in complex drug-environment interplay⁹⁷.

4. Expert Opinion

In order to improve the success of anxiolytic drugs in early clinical trials, the goals of preclinical trials may need to be adjusted from a clinical perspective, and better synchronized with those of clinical studies (Fig. 3). Indeed, one cannot expect the transition from one phase to another to be smooth if their strategic goals and approaches differ markedly (e.g., finding potent agents vs. ensuring low health risks or utilizing a short-term screens vs. long-term pharmacotherapy). Recognizing these challenges, the ISBS Task Force recommendations outlined here (also see Table 2) represent specific, translationally relevant strategies aimed at improving anxiolytic drug discovery, as well as drugs development and their transition to early clinical trials in particular.

Covering a wide spectrum of topics, these recommendations range from methodological improvements (e.g., automation, biomarker validation, dose/test/sex/age/strain selection) to conceptual insights (e.g., ‘clinization’, targeting disorder spectra and neurodevelopmental trajectories) and calls for further innovation of ASD research (e.g., by making a stronger focus on targeting novel pathways and applying imaging tools to ASD pathogenesis).

Declaration of Interest:

The authors have no conflict of interest.

Figure 1. Common anxiety spectrum disorders (ASDs) and other related stress-induced brain disorders (TSRDs). GAD – generalized anxiety disorder, GABA – gamma aminobutyric acid, SSRI – selective serotonin reuptake inhibitors, PTSD – post-traumatic stress disorder.

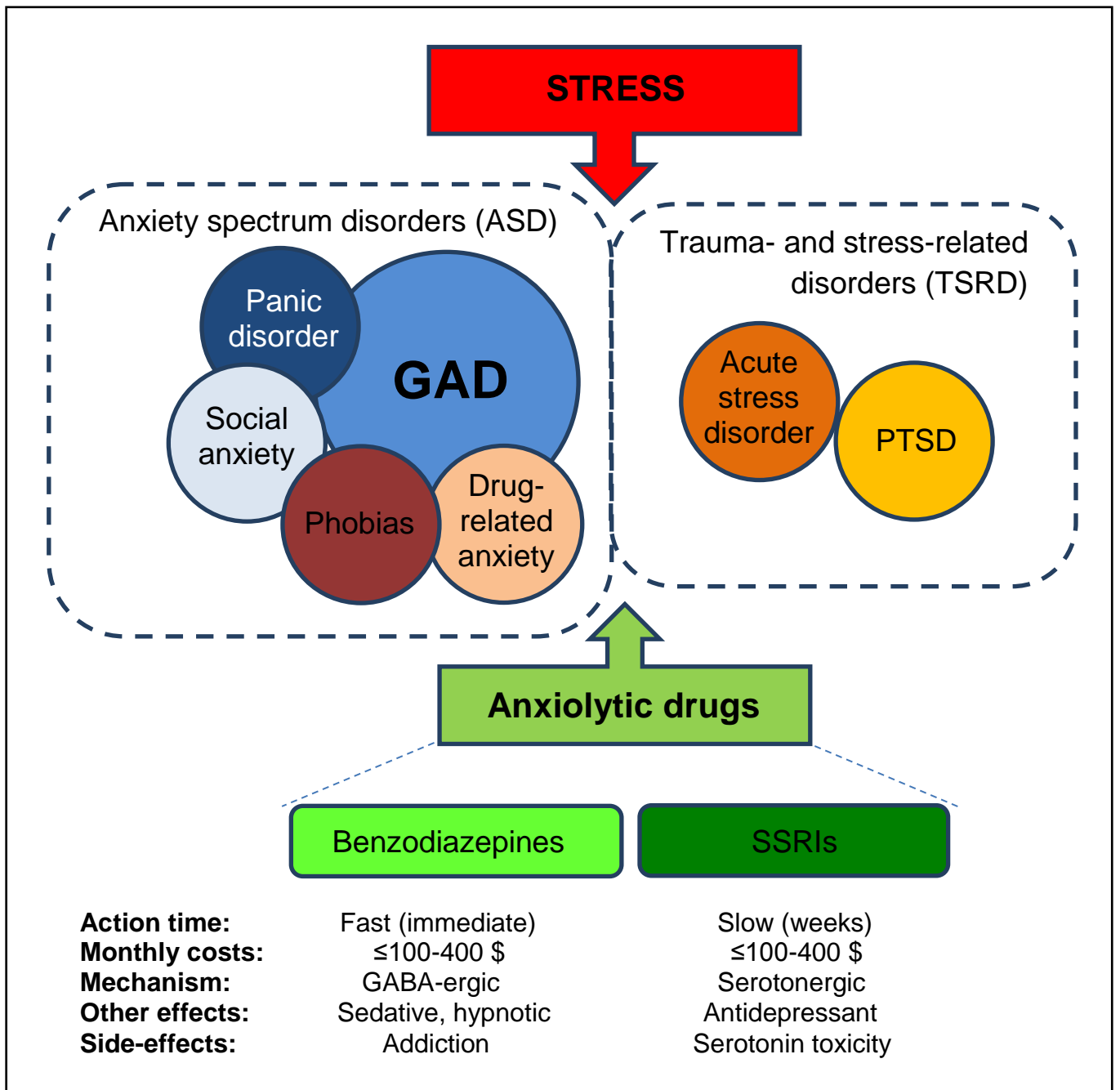


Figure 3. Inherent differences in strategic goals between preclinical and clinical trials

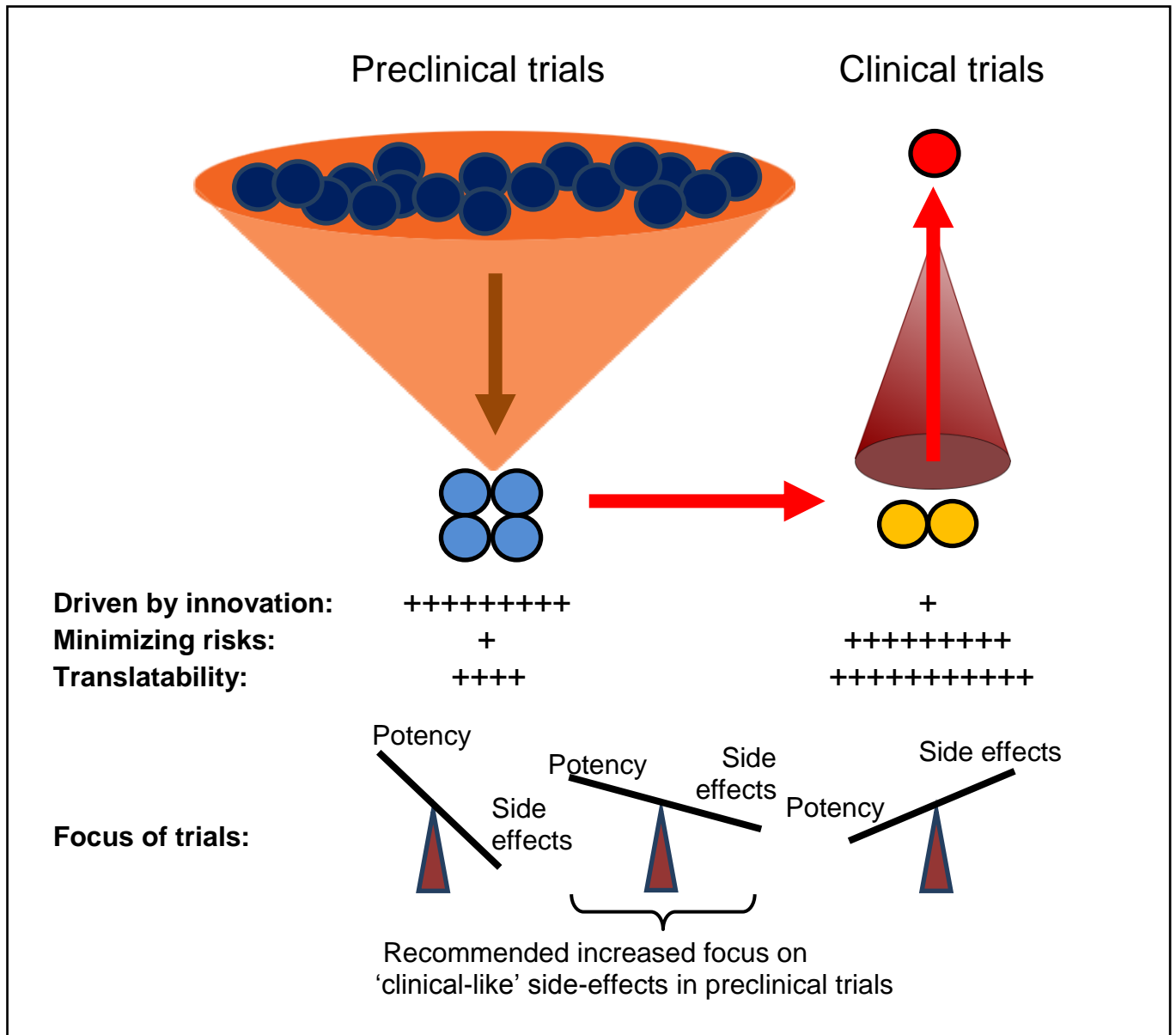


Figure 4. Perspectives on anxiolytic drug development based on both drug target and drug efficacy predictors. In addition to targeting the mechanism of anxiolytic drug action *per se* (e.g., via its receptor or transporter), it is important to ensure the drug efficacy. Thus, novel anxiolytic drugs can be developed based on targeting the drug efficacy predictors, in addition to drug receptors or transporters.

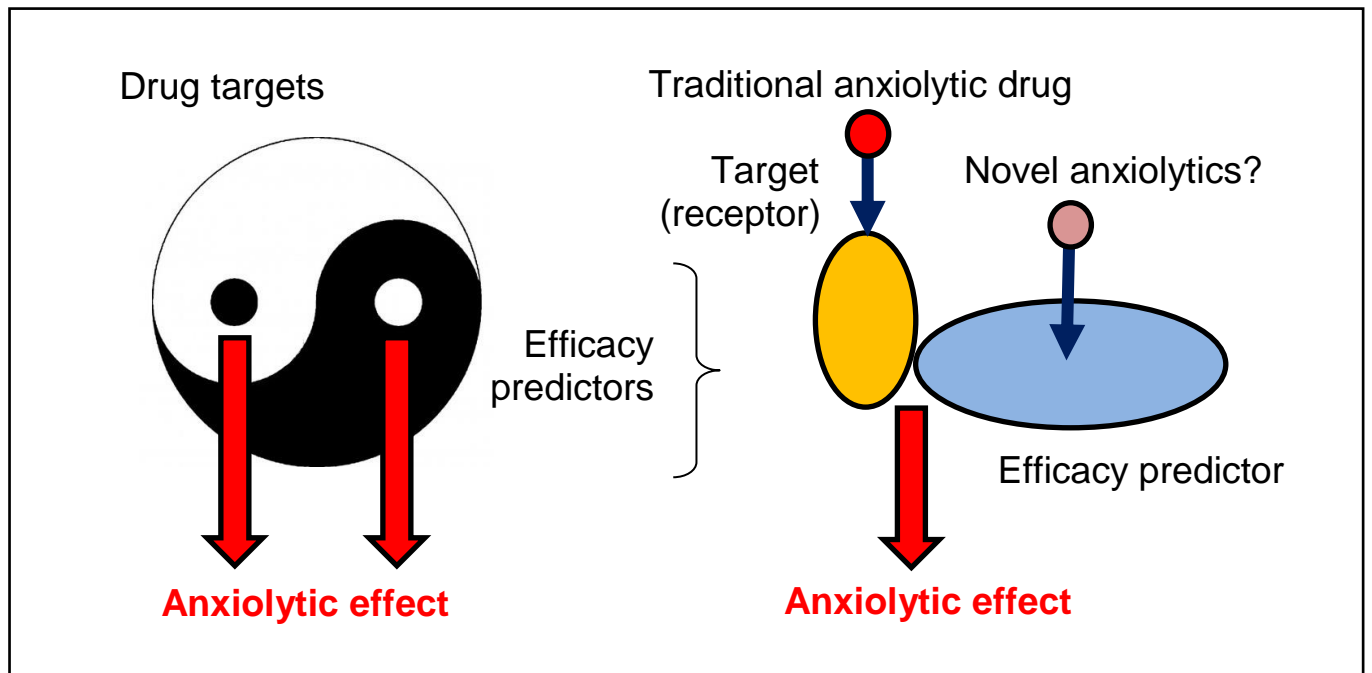


Table 1. Main classes of anxiolytic drugs (GAD – generalized anxiety disorder, ASD – anxiety spectrum disorders, PTSD – post-traumatic stress disorder, MDD – major depressive disorder)

Drug target	Drug examples	Disorders treated
GABA-ergic system	Benzodiazepines	GAD, other ASDs
Monoaminergic systems	Serotonin receptor ligands, SSRIs, tricyclic antidepressants (TCAs)	ASD, MDD, PTSD
Glutamatergic system	Glycine, D-serine	ASD
Endocannabinoid system	Nabilone (synthetic cannabinoid)	ASD/PTSD
Neuropeptides	MK869 (selective neurokinin 1 (NK1) receptor antagonist)	ASD, MDD
Glucocorticoid pathway	Synthetic antagonists	PTSD, MDD

Table 2. Selected additional strategies to improve anxiolytic drug development. ASD – anxiety spectrum disorder, GAD – generalized anxiety disorder, PTSD – post-traumatic stress disorders

Strategy	Details
Improve existing drugs	Improve drug activity via its known, established mechanism of action (e.g., by modifying the chemical structure to increase permeability, stability and/or receptor binding; see Table 1 for details)
Repurpose	Apply already established drugs for novel disorders/targets
Discover new mechanisms	Identify novel mechanisms of action for anxiolytic drugs (e.g., going beyond traditional benzodiazepine or serotonergic action)
Re-discover	Identify novel drugs acting via known, established mechanisms of action
Develop subset-specific drugs	Develop novel anxiolytics targeting anxiety in a disorder subtype-specific manner (e.g., anti-GAD, anti-panic, anti-withdrawal, anti-PTSD agents)
Develop sex- and age-specific drugs	Develop novel anxiolytics targeting anxiety in a subject-specific manner (e.g., drugs most active in geriatric anxiety; anxiolytics specifically targeting adolescents, male- vs. female-active anxiolytics)
Develop personalized medication	Develop novel anxiolytics based on individual patients' genetic and physiological risks and vulnerabilities (e.g., carriers of short/short alleles of the serotonin transporter <i>SERT</i> gene are generally resistant to therapeutic effects of SSRIs)
Develop poly-target medication	1) Develop novel anxiolytics based on combination of multiple pharmacological mechanisms (e.g., potential therapeutic effects of a hallucinogenic drug ibogaine and its analogs may include anxiolytic, anti-

	PTSD and anti-withdrawal action due to combined serotonergic, glutamatergic, cholinergic and opioidergic actions). 2) Develop anxiolytic drugs that have additional therapeutic properties that can indirectly contribute to alleviating ASD (e.g., using SSRIs as antidepressants can reduce depression, thereby minimizing overall stress of a subject, resulting in improved anxiety state; Similarly, mild anxiolytic amnestics can assist in blocking negative memories, thereby reducing PTSD symptoms by simultaneously decreasing anxiety and recurrent negative flashbacks)
Focus on additional disorders and their comorbidity	Develop anxiolytics that may work synergistically in combination with auxiliary drugs, such as anti-obesity or anti-stroke medication
Apply ‘big data’ approaches	Utilize ‘behavioral barcoding’ approaches*, which enable a fast clustering and visualization of ‘hidden’ drug-evoked patterns among multiple experimental conditions, drugs, dosages, and other factors
Examine physiological correlates	The inclusion of physiological (including peripheral) correlates of the disorder is consistent with the ‘systems biology’ approach. An interesting example is analyses of the expression of glucocorticoid receptor-related genes in peripheral blood in a rat model of PTSD**.
Apply sophisticated machine-learning algorithms	Utilize in-vivo screens with built-in machine learning algorithms able to extract pharmacological profiles of various drugs from multiple phenotypes by establishing target/mechanism-specific commonalities and differences in anxiolytic action
Expand the duration of behavioral tests	Although the majority of experimental ASD models is based on acute, short-term (typically 5-10 min, sometimes 30-60-min) testing protocols,

	<p>the actual clinical ASD-like states are much more protracted. Recognizing the importance of expanding experimental testing timeframe (in line with recent evidence^{21, 88}), we emphasize the value of longer-term monitoring of animal behavioral and related phenotypes in models of ASD and during anxiolytic drug testing.</p>
<p>Focus on potential placebo and nocebo (negative placebo) effects</p>	<p>Outcome prediction emerges as an important cognitive factor in the formation of placebo⁹⁷ – a factor that may modulate CNS drug efficacy. The same logic also applies to nocebo effects, collectively resulting in the need to better understand the placebo effects, their biological markers, relation to disease progression/risks, and associated molecular mechanisms. Determined by genetic factors and modulated environmentally through learning, placebo effects can lead to novel biological targets previously unrecognized in traditional drug development⁹⁷. We call for more preclinical research modeling placebo/nocebo responses in ASD, and examining the interplay of their biological pathways with those of anxiolytic drug targets.</p>
<p>Consider evidence generated by complementary and alternative medicine (CAM)</p>	<p>More research on the efficacy and safety of CAMs is needed, and the fields of ethnopharmacology and ethnobotany are importantly positioned to carefully and objectively evaluate potential anxiolytic effects of medicinal plants. Providing important ethical and cultural perspectives of healthcare and medicinal plants, these approaches can offer invaluable insights for both clinical and preclinical investigators⁹⁸.</p>
<p>Predict drugs' anxiolytic action by focusing on side-effects</p>	<p>While drug side effects are traditionally considered as unwanted, the negative information about the drug may be valuable in predicting its positive effects. For example, because many anxiolytics have shared types</p>

	<p>of well-established side-effects (e.g., sedation for benzodiazepines, serotonin toxicity for SSRIs), in-depth analyses of side effects of unknown compounds may be used to predict the drugs' potential pharmacological profile. For instance, unwanted serotonin syndrome-like behavior can suggest a likely serotonergic profile of the putative anxiolytic agent.</p>
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* See ⁹⁹⁻¹⁰¹ for recent excellent examples of using behavioral barcoding in animal preclinical studies.

** 7 days after a predator scent exposure (see ¹⁰² for details).

Table 3. A wide spectrum of treatment regimens used clinically, which can be more routinely applied to preclinical screening procedures, to complement the traditional acute and drug chronic administration

Factor	Additional treatment regimens
Treatment initiation time	Early (pre-, neo, post-natal) Late (in older, aged, very old age)
Treatment duration	Sub-chronic
Treatment frequency	Repeated Intermittent Continuous
Effect evaluation time	Delayed response (e.g., effect evaluation several weeks later) Developmental effects (e.g., treatment early, analyses in adults) Trans-generational

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